SUCCESSFUL CULTIVATION OF GONOCOCCUS IN TWO CASES OF GONORRHEAL ARTHRITIS AND ONE OF TÆNOSYNOVITIS, WITH REMARKS ON A NEW MEDIUM.

By Francis R. Hagner, M. D.,
Assistant Resident Surgeon, Johns Hopkins Hospital.

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The more successful attempts to cultivate the gonococcus from pathological conditions other than urethritis and conjunctivitis, have widened our view concerning the part played by this organism in human pathology.

Even now it would take much space to enumerate all the different lesions and parts of the body in which this organism has been found. The gonococcus, as is well known, cannot be cultivated with the facility of the other pyogenic cocci, and it has for this reason not lent itself so readily as an aid to diagnosis in obscure cases, unless perchance it could be found in cover-slips, where its peculiar form and definite staining reaction might suffice for its identification.

Greater experience, on the other hand, has shown that, although this organism does not grow at all, or at least most feebly and unsatisfactorily, upon the ordinary culture media, its choice of substance is still not a small one.

Since Steinschneider's observation of the great value of urine in the composition of a culture medium for the gonococcus, no really easy practicable method of preparing a medium containing this fluid has been devised.

We offer in the accompanying paper a simple method which, in the limited number of cases at our disposal, has proven successful.

It is in part my object in presenting these cases to draw attention to the ease with which, by the use of this medium, the gonococcus may be cultivated.

I must state that the value of the medium was further

tested and proven by cultivating this micro-organism from urethral pus. The cases themselves are of interest from their clinical aspects, and as illustrating the good results which surgical interference gives when undertaken in time.

The possibility of making a positive diagnosis before opening the infected joint enhances the likelihood of good which may be confidently expected from these measures.

No question is likely to arise as to the identity of the cocci isolated in these cases, even in view of the fact that in one case the organism did not completely decolorize when treated according to Gram's method.

The later writers on the subject, among whom I shall only mention Caplewski,* concede great difference in the behavior of cocci from different sources.

Most samples of gonococci are quickly and readily decolorized; some few are more refractory and may retain the stain in part. On the other hand, the ordinary pyogenic cocci which resist Gram's method sometimes become decolorized.

No small part in this procedure is played by the composition of the stain and decolorizing agents.

But when all the facts are gathered, namely, the source of the organisms, their morphological properties, their difficulty of culture, and slight viability, together with their staining reaction, no doubt is likely to be entertained concerning their nature.

Case I.

B. B., æt. 21, female, colored. Domestic. Admitted December 5, 1896.

Previous History. Patient has never been very healthy. One year ago she had an attack of rheumatism; at this time the right knee was swollen and painful. The patient was confined to bed for one month, and has never had any trouble with the joint since. There was no history of any vaginal discharge at this time.

Present Illness. Patient acknowledges exposure within the last month. Has had vaginal discharge for three weeks. Six days before entrance to the hospital she noticed pain and

*Hygienische Rundschau, Vol. 6, No. 21, p. 1029.

swelling of the left knee. Pain more marked at night and increased by motion.

Examination. The patient is a rather poorly nourished, unintelligent woman, with a slight blowing murmur over the apex of the heart, transmitted to axilla. The joint is quite tense, painful on palpation and motion. Patella floats. There is marked induration and thickening of the peri-articular tissues, which are boggy. Distinct fluctuation over the joint. A purulent discharge observed to be present in vagina and urethra. The examination of cover-slips was negative for typical gonococcus-like organisms.

December 5th. Knee aspirated with sterile syringe and a straw-colored fluid obtained; this showed under the microscope a great many polymorphonuclear leucocytes, and a few large diplococci which were not contained in the pus cells. Cultures made on agar-agar, gelatin, potato and bouillon were negative after forty-eight hours in the thermostat. Cultures made at the same time on albuminous urine agar in twenty-four hours showed no perceptible growth, but at the end of forty-eight hours in the thermostat about a dozen isolated colonies, a little larger than ordinary streptococcus colonies, elevated above the surface of the medium, presenting an opaque white color, but still translucent, were easily seen.

Cover-slips were made, stained with Sterling's gentian violet, mounted in water. The examination showed diplococci morphologically identical with the gonococcus. The same specimens were then stained by Gram's method and almost completely decolorized, a faint outline still being visible here and there. The ordinary media (mentioned previously) were inoculated from the cultures with negative results; but another albuminous urine agar tube, inoculated, gave a similar growth to the first after forty-eight hours in the thermostat, and this showed the same morphological characteristics.

Another generation, third in succession, was obtained on the albuminous urine agar medium; this one was feebler than the preceding ones, and no further growth was obtainable.

December 8th. Knee again aspirated, the fluid giving the same growth when inoculated on the albuminous urine agar tube.

The same negative results as described previously were

obtained on the ordinary media. The growth mentioned was carried through three generations, but again would not grow on the fourth transplantation.

December 17th. The knee joint was opened and cultures taken, two tubes of the albuminous urine agar being inoculated. One of these became contaminated; in the other the growth was very slight and did not survive for a second transplantation.

The following is a brief description of the mode of preparation of the albuminous urine agar, which was prepared by Dr. Hugh Young and myself.

Acid urine containing 0.05 albumen or more should be collected and allowed to stand for twenty-four hours, no effort being made to prevent decomposition. The urine is boiled until a large albuminous precipitate is formed; it is filtered through paper, when the resulting fluid will be clear. The filtered urine is boiled, and agar-agar, peptone, beef extract and sodium chloride are added in the same proportion as making ordinary agar.

The other steps are the same as in making ordinary agar, except that filtered albuminous urine instead of water is used throughout the preparation of the medium. It is important to see that the medium before being placed in tubes has a neutral or slightly acid reaction.

The advantages of using albuminous urine are, first, that in such urine albumens are always present, which are not coagulated by heat, and second, the albumen that is coagulated acts as a clarifying agent in the removal of the salts that usually cause the cloudiness of urine agar-agar as prepared by mixing the urine agar separately and sterilizing by discontinuous heating below the point of coagulation. It is important to have the medinm very moist when inoculated.

The operation consisted of opening and irrigating with bichloride of mercury 1 to 1000, followed by salt solution, an Esmarch bandage being applied above the joint to prevent absorption of the bichloride solution.

The wound was approximated with subcutaneous silver wire sutures, silver foil dressing applied, and the leg put up in plaster. Of course very strict cleanliness is necessary in these operations—in all cases the operator and assistants wearing rubber gloves. The wound healed *per primam.*

At the time of operation the subcutaneous tissues were found very œdematous and thickened, and minute hemorrhagic areas were seen in the tissues near the joint. The fluid within the joint was serous in character, although flakes of fibrin were contained in it. The synovial membrane was thickened and its surface covered with hemorrhagic material that in places had a plush-like appearance, having lost its gloss. At the junctions of the cartilages and synovial membrane there were a number of tessellated, very vascular fringes of fibrinous material 3 to 5 mm. in length.

The cartilages showed no change.

The patient is at present more comfortable, but has not entirely recovered.

CASE II.

A. D., female, æt. 20 years, colored, domestic. Admitted August 25, 1895.

No history of rheumatism.

Has had vaginal discharge for two weeks. (Patient acknowledges exposure several days before the discharge was noted.)

Three days before entrance left knee joint became painful and swollen, pain being more marked at night; fever was present, the highest temperature recorded being 103° F.

Examination. Large, well nourished woman.

Left knee slightly flexed, and warmer than adjacent parts. Slight fluctuation on inner side of patella; movement of the affected joint caused great pain.

The peri-articular tissues were indurated and boggy.

There was a purulent discharge from the vagina and urethra that contained diplococci. These were in a manner suggestive of the gonococcus and occurred within the pus cells; they completely decolorized when stained according to Gram's method.

The operation was done on the fifth day of the disease, and consisted in the application of an Esmarch bandage, incision of the joint, irrigation with 1 to 1000 bichloride of mercury followed by salt solution, and closure of wound with silver wire. Silver foil dressing and plaster cast applied.

Patient made good recovery.

The examination of joint at time of operation showed the

peri-articular tissues to be in an œdematous and hemorrhagic condition.

The joint contained about 25 cc. of blood-stained fluid in which floated small pieces of a fibrinous material.

The synovial membrane was roughened, thickened and had the same appearance described in the preceding case.

Larger tessellated masses of fibrin adhered to synovial membrane wherever it came in contact with the cartilage.

Bacteriological Examination.

The fluid for culture was removed from the joint with a sterile Volkman spoon, and placed in sterile test tube. A small quantity of blood was obtained by allowing a stream from a small artery to spurt into a sterile test tube.

The tube containing the blood was allowed to stand for two hours, during which time the serum had separated from the clot and could be pipetted off. An ordinary agar tube was melted and cooled to 46° C., so as to prevent the blood serum from coagulating when added. About 5 cc. of the human blood serum was added, making the proportion one-third human blood serum and two-thirds nutrient agar-agar; the resulting medium was perfectly clear. The fluid medium was then mixed thoroughly, and inoculated with three loops of fluid obtained from the joint, great care being taken not to add the fluid until the medium was observed to be on the point of solidifying, so as to prevent all chances of destruction of the organism by heat.

The inoculated medium was poured into a Petri's dish and placed in thermostat at 37° C. No growth was visible at the end of the first twenty-four hours, but at the expiration of forty-eight hours five or six small colonies could be seen. These were isolated and about the size of the ordinary streptococcus colonies, but they were more elevated when they appeared on the surface of the medium, and of a more opaque white color; they were, however, slightly translucent.

Cover-slips prepared from such a colony and stained with Sterling's gentian violet, mounted in water, showed numerous diplococci somewhat larger than the ordinary pyogenic cocci, composed of two hemispheres separated by a narrow unstained interval; a few tetrad forms were also seen. The same preparation treated by Gram's method was completely decolorized.

Agar-agar, bouillon, potato, gelatin, and glycerine-agar were then inoculated from one of the colonies.

At the same time another culture was made on the serum agar. No growth could be seen after forty-eight hours on any of the tubes except the one containing the human serum agar, and on this a growth similar in appearance to the ones described before, consisting of cocci with the same morphological properties, was found; further transplantation was not successful on this medium.

As no perceptible growth occurred on any of the ordinary cultural media, and cover-slips taken from their surfaces were negative, the conclusion that the organism was the gonococcus was considered justified.

It is interesting to note that although numerous cover-slips were made from the fluid at the time of operation, and numbers of polymorphonuclear leucocytes were found, no micro-organisms could be discovered.

Case III.

A. F., male, white, single, 39 years. Admitted May 20, 1896.

Denied any venereal disease. (Very questionable.)

Patient felt, without any premonitory symptoms, great pain in the left ankle joint, and at the same time noticed that there was considerable swelling and redness of the skin over the joint.

The pain was more marked at night, and increased with movement.

The condition mentioned gradually grew worse until the twenty-fourth day after the beginning of the disease, when patient was transferred to the surgical ward.

Examination. Patient was a well nourished man. Temperature on entrance 100° F. There was a fluctuating swelling extending from the juncture of middle and lower third of tibia, following the sheaths of extensor muscles, to a point on the dorsum of foot 3 cm. below the ankle joint.

May 21st, Operation. Same operation as described previously.

Incision of abscess and excision of fibrinous material from tendon sheaths. Irrigation of bichloride of mercury 1 to 1000,

wounds closed with silver wire and dressed with silver foil, and leg put up in plaster. Patient made good recovery in three weeks, wound healing *per primam.* On incision the subcutaneous tissues were œdematous and slightly hemorrhagic. The tendon sheaths were thickened and covered with hemorrhagic fibrinous material.

The pus was confined principally to the sheaths of tibialis anticus and extensor proprius pollicis, chiefly about the annular ligament, but followed the pollicis to a distance of 3 cm. below. The sheaths were opened and about 100 cc. of blood-stained fluid escaped, which was placed by means of a Volkman spoon in a sterile test tube.

The internal portions of the sheaths were covered with a hemorrhagic fibrinous material and some granulation tissue.

I am indebted to Dr. Flexner for the privilege of reporting his successful cultivation of the gonococcus in this case. The pus collected at operation in a sterile manner was sent to the Pathological Laboratory.

Cover-slips when stained with Sterling's gentian violet showed polymorphonuclear leucocytes filled with diplococci morphologically resembling the gonococcus; a few of the organisms seen were extra-cellular.

When stained according to Gram's method the organisms were completely decolorized. Inoculations of the pus were made on the mixture of Steinschneider,* on a mixture composed of human ascitic fluid and agar-agar,† on a mixture of human blood serum and urine,‡ on an infusion of pig-fœtuses and nutrient agar,§ and also upon ordinary agar slants. The

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*Steinschneider's medium consists of a mixture of bullock's serum, urine, and agar-agar.

† The mixture of ascitic fluid $\frac{1}{3}$ and agar-agar $\frac{2}{3}$, which after being placed in tubes is sterilized and slanted. An albuminous flaky precipitate collects at the bottom of the medium, leaving surface clear.

‡ Human blood serum and urine medium is composed of $\frac{1}{3}$ urine, $\frac{2}{3}$ human blood serum sterilized in autoclave at 220° F. (Human serum derived from placenta.)

§ *Preparation of pig-fœtus agar:* Fresh pig-fœtuses not exceeding 5 cm. in length separated from placenta and membranes are minced in a sausage machine. An equal volume of distilled water is added to the finely divided fœtuses, and after thoroughly

cultures were placed in a thermostat at 37° C., and at end of twenty-four hours a scarcely perceptible growth was found on all the inoculated tubes except the agar slants, which last remained sterile, whereas the growth on the other tubes increased somewhat during the next twenty-four hours.

The appearance of the growth was the same as that described in previous cases.

Growth on pig-fœtus agar was more abundant and apparently more vigorous than on the other media.

Cover-slips from the cultures showed the same diplococcus as was found in the pus, and it became decolorized completely by Gram's method.

Transplantations at intervals of forty-eight hours were made on pig-fœtus medium mentioned and growth obtained for four generations, but from the fifth inoculation no growth resulted.

It is interesting to note that the condition of synovial membranes and peri-articular tissues in these cases was practically the same, namely subcutaneous œdema, thickening and induration of peri-articular tissues, with small hemorrhagic areas.

The synovial membrane was thickened, very hemorrhagic and had the appearance of plush, having lost the glossy condition.

The fringe-like pieces of fibrin were very hemorrhagic. In neither case was the cartilaginous portion of joint affected.

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stirring, the mixture is allowed to macerate in a cool place for from six to twelve hours. The fluid is then freed from contamination by filtration through a Chamberland filter under a pressure of 150 to 200 lbs.

Two per cent. sterilized nutrient agar is then melted and cooled to 40° C. and to it ⅛ of its volume of the infusion of fœtuses is added. The tubes are then slanted.